REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 µL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 µm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

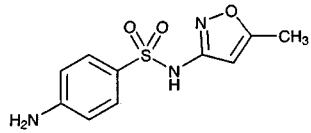
CHROMATOGRAM
Retention time: 5.3
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfathiazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

Sulfamethoxazole



Molecular formula: $C_{10}H_{11}N_3O_3S$
Molecular weight: 253.28
CAS Registry No.: 723-46-6
Merck Index: 9086

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 µL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 µm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.42

OTHER SUBSTANCES
Extracted: ethosuximide, methamphetamine, primidone

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fritz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.445

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphet-

amine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 46

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 42

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 16
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS

fish; salmon; post-column reaction

REFERENCE

Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE

Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 7.7
Internal standard: niacin (3.3)

OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamerazine, sulfamonomethoxine

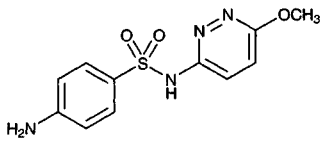
KEY WORDS

wastewater

REFERENCE

Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethoxypyridazine



Molecular formula: $C_{11}H_{12}N_4O_3S$
Molecular weight: 280.31
CAS Registry No.: 80-35-3, 2577-32-4 (sodium salt)
Merck Index: 9087

SAMPLE

Matrix: milk
Sample preparation: Mix 5 mL milk with 20 μL 12 M HCl, sonicate, add 25 mL ethyl acetate, extract using a rotary shaker (REAX 2, Heidolph) for 10 min. Centrifuge at 1500 g for 5 min, evaporate 20 mL of the ethyl acetate extract to dryness, dissolve the residue in 10 mL 1 HCl. Wash the aqueous phase with 10 mL dichloromethane, adjust to pH 5.5 with 900 μL 10 M NaOH and 5 mL 1 M pH 6.0 KH_2PO_4, extract with two 10 mL portions of dichloromethane. Evaporate the organic layer to dryness, dissolve the residue in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Novapak C18 (Waters)
Mobile phase: MeCN:10 mM pH 6.6 ammonium acetate 10:90
Flow rate: 1
Injection volume: 50
Detector: UV 265

CHROMATOGRAM

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Also analyzed: sulfadimethoxine (UV 271), sulfadimidine, sulfadoxine (UV 271)

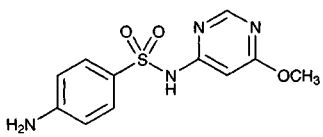
KEY WORDS

cow; milk

REFERENCE

Roudaut,B.; Moretain,J.P. Sulphonamide residues in milk of dairy cows following intravenous injection, *Food Addit.Contam.*, **1990**, *7*, 527–533.

Sulfamonomethoxine



Molecular formula: $C_{11}H_{12}N_4O_3S$
Molecular weight: 280.31
CAS Registry No.: 1220-83-3

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood. Filter serum through a 0.45 μm syringe filter with a cellulose acetate membrane, inject a 50 μL aliquot of the filtrate. Tissue. Add 1 mL MeCN:THF 95:5 to 1 g muscle, homogenize with a Pencil Mixer (Iuchi, Japan) for 2 min, centrifuge at 1500 g for 5 min, filter the supernatant through a syringe filter unit, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μL Hisep shielded hydrophobic phase precolumn (Supelco)
Column: 150 × 4.6 5 μL Hisep shielded hydrophobic phase (Supelco)
Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM citric acid:200 mM pH 2.5 Na_2HPO_4 buffer containing 10 mM tetra-*n*-butyl ammonium bromide 85:15.)

Flow rate: 1
Injection volume: 20-50
Detector: UV 265

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 50 ng/mL (serum), 100 ng/mL (muscle)

OTHER SUBSTANCES
Extracted: oxolinic acid, miloxacin

KEY WORDS
fish; muscle; serum

REFERENCE
Ueno,R.; Aoki,T. High-performance liquid chromatographic method for the rapid and simultaneous determination of sulfamonomethoxine, miloxacin and oxolinic acid in serum and muscle of cultured fish, *J.Chromatogr.B*, **1996**, *682*, 179–181.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

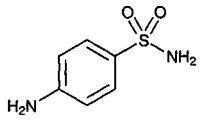
CHROMATOGRAM
Retention time: 9
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamerazine, sulfamethoxazole

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfanilamide



Molecular formula: $C_6H_8N_2O_2S$
Molecular weight: 172.21
CAS Registry No.: 63-74-1
Merck Index: 9094
Lednicer No.: 1 121

SAMPLE

Matrix: blood
Sample preparation: Keep tubes in crushed ice except when being processed throughout this procedure. 2 mL Plasma + 8 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Remove ether layer and add it to 1 mL 100 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 1 mL 100 mM HCl and 500 μL 50 mM pH 7.4 sodium phosphate, add 8 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:100 mM pH 3.6 sodium acetate buffer 43:57
Column temperature: 54
Flow rate: 1
Injection volume: 50
Detector: UV 241

CHROMATOGRAM

Retention time: 5.3
Internal standard: sulfanilamide

OTHER SUBSTANCES

Simultaneous: indapamide

KEY WORDS

plasma; sulfanilamide is IS

REFERENCE

Choi,R.L.; Rosenberg,M.; Grebow,P.E.; Huntley,T.E. High-performance liquid chromatographic analysis of indapamide (RHC 2555) in urine, plasma and blood, *J.Chromatogr.*, **1982**, *230*, 181–187.

SAMPLE

Matrix: blood, urine
Sample preparation: Keep tubes in crushed ice except when being processed throughout this procedure. Blood. 1 mL Blood + 4 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Repeat extraction, combine ether layers, add 500 μL 10 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 500 μL 10 mM HCl and 250 μL 50 mM pH 7.4 sodium phosphate, add 4 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μL mobile phase, inject 50 μL aliquot. Urine. 1 mL Urine + 4 mL diethyl ether, vortex for 2 min, centrifuge at 4°. Repeat extraction, combine ether layers, add 500 μL 50 mM NaOH, vortex for 2 min, centrifuge at 4°. Remove aqueous layer and add it to 500 μL 50 mM HCl and 250 μL 50 mM pH 7.4 sodium phosphate, add 4 mL ether, vortex for 2 min, centrifuge at 4°. Evaporate ether layer to dryness, reconstitute in 200 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.2 10 μm LiChrosorb C-18
Mobile phase: MeCN:100 mM pH 3.6 sodium acetate buffer 35:65
Column temperature: 54
Flow rate: 1.5
Injection volume: 50

Detector: UV 241

CHROMATOGRAM
Retention time: 2.5
Internal standard: sulfanilamide

OTHER SUBSTANCES
Simultaneous: indapamide

KEY WORDS
sulfanilamide is IS

REFERENCE
Choi,R.L.; Rosenberg,M.; Grebow,P.E.; Huntley,T.E. High-performance liquid chromatographic analysis of indapamide (RHC 2555) in urine, plasma and blood, *J.Chromatogr.*, **1982**, *230*, 181–187.

SAMPLE
Matrix: cell suspensions
Sample preparation: Cool cell suspension in an ice bath, centrifuge at 800 g at 4° for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Also analyzed: sulfadiazine, sulfamethoxazole, sulfamerazine

REFERENCE
Climax,J.; Lenehan,T.J.; Lambe,R.; Kenny,M.; Caffrey,E.; Darragh,A. Interaction of antimicrobial agents with human peripheral blood leucocytes: uptake and intracellular localization of certain sulphonamides and trimethoprims, *J.Antimicrob.Chemother.*, **1986**, *17*, 489–498.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column

A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 1.0

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

Interfering: sulfacetamide, sulfaguanidine

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Homogenize 3 g milk and 500 μL 30% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot. Fish, eggs. Homogenize (Ultra-Turrax) 3 g fish or 4 g eggs with 4 mL 3% trichloroacetic acid, centrifuge at 5000 rpm for 5 min. Remove the aqueous phase and extract the residue with 4 mL 3% trichloroacetic acid. Combine the aqueous layers and make up to 10 mL with trichloroacetic acid, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Spherisorb ODS-2

Column: 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: Gradient. MeCN:water 3:97 for 5 min, to 40:60 over 15 min, return to initial conditions over 1 min, re-equilibrate for 10 min. (At the end of each day wash with MeCN:ethyl acetate 5:95.)

Flow rate: 0.5

Injection volume: 50

Detector: F ex 302 em 412 following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and with reagent 2 pumped at 0.25 mL/min and this mixture flowed through a 2.5 m × 0.8 mm i.d. PTFE coil at 40° to the detector. (Reagent 1 was 10 mM o-phthalaldehyde in EtOH:700 mM phosphoric acid 2:98. Reagent 2 was 20 mM β-mercaptoethanol in EtOH:700 mM phosphoric acid 2:98.)

CHROMATOGRAM

Retention time: 10

Limit of detection: 18 ng/mL

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfapyridine

Noninterfering: sulfathiazole

KEY WORDS
post-column reaction; derivatization

REFERENCE
Viñas,P.; Erroz,C.L.; Campillo,N.; Hernández-Córdoba,M. Determination of sulphonamides in foods by liquid chromatography with postcolumn fluorescence derivatization, *J.Chromatogr.A*, **1996**, *726*, 125–131.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Suspension + 100 mL MeOH:water 60:40, shake mechanically for 15 min, make up to 200 mL with MeOH:water 60:40, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 1 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL 200 μg/mL acetanilide in MeCN, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: acetanilide (11)

OTHER SUBSTANCES
Simultaneous: sulfadiazine, sulfamerazine, sulfamethazine, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: formulations
Sample preparation: Weigh capsule contents, dissolve in 50 mL MeOH, add 5 mL 0.75 mg/mL sulfanilamide in MeOH, make up to 100 mL with MeOH, filter (0.45 μm), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC8DB C8
Mobile phase: MeOH:1% acetic acid 40:60
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 0.8
Internal standard: sulfanilamide

OTHER SUBSTANCES
Simultaneous: temazepam

KEY WORDS
capsules; sulfanilamide is IS

REFERENCE
Fatmi,A.A.; Hickson,E.A. Determination of temazepam and related compounds in capsules by high-performance liquid chromatography, *J.Pharm.Sci.*, **1988**, 77, 87–89.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 11
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfathiazole
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: formulations, bulk
Sample preparation: Dilute with water to an idoxuridine concentration of 0.1%. Remove a 16 mL aliquot and add it to 2 mL 0.001% sulfanilamide in water, make up to 20 mL with water, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 4:96
Flow rate: 1.7
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: sulfanilamide

OTHER SUBSTANCES
Simultaneous: idoxuridine

KEY WORDS
eye drops; sulfanilamide is IS

REFERENCE
Carr,G.P.R. The development of British Pharmacopeia monographs for idoxuridine eye drops using high-pressure liquid chromatography for assay and for controlling related impurities, *J.Chromatogr.*, **1978**, *157*, 171–184.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 1.2
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfathiazole, sulfadiazine, sulfamethazine, sulfisoxazole, sulfadimethoxine

KEY WORDS
matrix solid-phase dispersion

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 3.1

Limit of detection: 4.9 ppb
Limit of quantitation: 9.1 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfapyridine, sulfathiazole

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100: 0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
cow

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: perfusate
Sample preparation: 1 mL Perfusate + 1 mL 1 M phosphoric acid + 10 mL ethyl acetate:isopropanol 90:10, vortex for 3 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 150 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 4 μm Novapak phenyl in a Z-module radial compression module
Mobile phase: MeOH:buffer 4:96 (Buffer was 25 mM K_2HPO_4 + 5 mM tetrabutylammonium + 5 mM triethylamine, pH adjusted to 6.8 with concentrated phosphoric acid.)
Flow rate: 3
Injection volume: 20

Detector: UV 264

CHROMATOGRAM
Internal standard: sulfanilamide

OTHER SUBSTANCES
Extracted: acipimox

KEY WORDS
sulfanilamide is IS

REFERENCE
Ghabrial,H.; Czuba,M.A.; Stead,C.K.; Smallwood,R.A.; Morgan,D.J. Transfer of acipimox across the isolated perfused human placenta, *Placenta.*, **1991**, *12*, 653–661.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfapyridine, sulfisoxazole

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfadiazine, sulfapyridine, sulfamerazine, sulfamethizole, sulfamethazine, sulfamethoxazole, sulfisoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 90 μL aliquot of a solution in MeOH:water 20:80.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:glacial acetic acid 10:89:1
Flow rate: 1.5
Injection volume: 90
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: sulfacetamide

REFERENCE
Hall,L.; Chadwick,V. Quantitative determination of sulfanilamide in sodium sulfacetamide raw material and ophthalmic solutions by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *478*, 438–445.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilic acid, sulfathiazole, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 3.74

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 3.0

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-

diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, succinylsulfathiazole

REFERENCE

Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 5 µm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.8

Injection volume: 50

Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM

Retention time: 3.75

Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES

Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfapyridine, sulfathiazole

KEY WORDS

post-column reaction; muscle; kidney; SPE

REFERENCE

Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 µL 10 µg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 µL MeOH:water 50:50, filter (0.45 µm), inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM

Retention time: 3
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES

Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS

post-column reaction; muscle; liver; kidney; SPE

REFERENCE

Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 4

Limit of quantitation: 5 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine diluted 1:10 + 50 μL MeOH + 100 μL 0.5 M HCl + 100 μL 0.1% sodium nitrite in water, vortex, let stand for 10 min, add 100 μL 2% ammonium sulfamate in water, let stand for 15 min, add 100 μL 0.05% 2-aminoanthracene in MeCN (Caution! 2-Aminoanthracene causes cancer in experimental animals!), let stand for 15 min in the dark, add 5 mL diethyl ether, shake for 5 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 300 μL MeOH, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm YMC-Pack A-312 (YMC)
Mobile phase: MeOH:water:acetic acid 78:22:1
Flow rate: 1
Injection volume: 30
Detector: UV 279

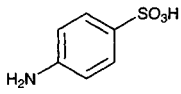
CHROMATOGRAM
Retention time: 8
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: p-aminobenzoic acid, 4-aminobenzoyl-β-alanine

KEY WORDS
derivatization

REFERENCE
Hayashi,T.; Amino,M.; Uchida,G.; Sato,M. High-performance liquid chromatographic determination of primary aromatic amines in urine after derivatization to an azo dye with 2-aminoanthracene, *J.Chromatogr.B*, **1995**, *665*, 209–212.

Sulfanilic acid



Molecular formula: $C_6H_7NO_3S$
Molecular weight: 173.19
CAS Registry No.: 121-57-3, 6101-32-2 (H2O), 6106-22-5 (sodium salt 2.H2O), 31884-76-1 (zinc salt 4.H2O)
Merck Index: 9096

SAMPLE
Matrix: solutions

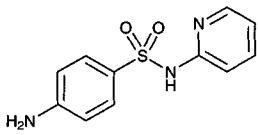
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 67.5:32.5 (Prepare mobile phase by dissolving 784 mg K_2HPO_4 in 325 mL water. Dissolve 2.62 g hexadecyltrimethylammonium bromide in 350 mL MeOH. Combine solutions, add 325 mL MeOH, mix.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: nalidixic acid

REFERENCE
Walker,S.T. Liquid chromatographic determination of nalidixic acid in pharmaceutical preparations, *J.AOAC Int.*, **1996**, *79*, 431–433.

Sulfapyridine



Molecular formula: $C_{11}H_{11}N_3O_2S$
Molecular weight: 249.29
CAS Registry No.: 144-83-2, 127-57-1 (Na salt monohydrate)
Merck Index: 9108
Lednicer No.: 1 124

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (A) or MeCN:20 mM pH 6.9 phosphate buffer 20:80 (B)
Flow rate: 1
Injection volume: 20 (A), 5 (B)
Detector: UV 254

CHROMATOGRAM
Retention time: 14.5 (A), 2.06 (B)

OTHER SUBSTANCES
Extracted: acetaminophen (A), barbital (A), carbamazepine (A,B), phenobarbital (A), phenytoin (A), primidone (A)

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fritz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood

Sample preparation: Extract 250 μL plasma with 1 mL 1 M pH 4.7 sodium acetate and 5 mL chloroform (Caution! Chloroform is a carcinogen!), evaporate to dryness under nitrogen at 40°, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco LC-8
Mobile phase: MeOH:50 mM pH 7 sodium dihydrogen orthophosphate 16:84
Flow rate: 1.3
Detector: UV 260

CHROMATOGRAM
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Awni; W.M.; Braeckman; R.A.; Locke; Ch.S.; Dubé; L.M.; Granneman; G.R. The influence of multiple oral doses of zileuton on the steady-state pharmacokinetics of sulfasalazine and its metabolites, sulfapyridine and *N*-acetylsulfapyridine, *Clin.Pharmacokinet.*, **1995**, *29*, 98–104.

SAMPLE
Matrix: saliva
Sample preparation: 1 mL Saliva + 148 ng sulfadiazine + 1 mL MeCN + 400 mg potassium carbonate, vortex for 1 min, centrifuge at ≥1000 g for 10 min. Remove the upper MeCN layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM $NaHPO_4$ (sic) containing 10 mM sodium 1-hexanesulfonate and 7.2 mM triethylamine, adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: F ex 395 em 470 following post-column reaction. The column effluent mixed with reagent pumped at 0.3 mL/min and the mixture flowed through a 4.8 m × 0.7 mm ID PTFE coil at 60° to the detector. (Prepare reagent by dissolving 400 mg fluorescamine in 250 mL MeOH, add 1 mL 2-mercaptoethanol, add 250 mL mobile phase.)

CHROMATOGRAM
Retention time: 7.32
Internal standard: sulfadiazine (5.66)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: N-acetylsulfapyridine, 2-amino-3-phenyl-1-propanol, 5-aminosalicylic acid, amphetamine, furosemide, levallorphan, metoprolol, riboflavin, salicylic acid, sulfasalazine, viloxazine

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KEY WORDS
post-column reaction

REFERENCE
Sista,H.S.; Dye,D.M.; Leonard,J. High-performance liquid chromatographic method for determination of sulfapyridine in human saliva using post-column, in-line derivatization with fluorescamine, *J.Chromatogr.*, **1983**, *273*, 464–468.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

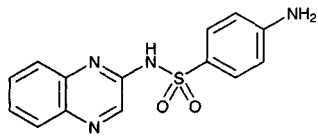
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Sulfaquinoxaline



Molecular formula: $C_{14}H_{12}N_4O_2$
Molecular weight: 300.34
CAS Registry No.: 59-40-5
Merck Index: 9109

SAMPLE

Matrix: blood, tissue
Sample preparation: Slurry 6 g alumina (alumina B Akt. I, ICN Biomedicals) in MeCN:MeOH 60:40, add to a 300 × 15 column, wash with 30 mL MeCN:MeOH 60:40. Homogenize (Niti-on Bio-mixer BM-2) 5 g chopped tissue or plasma with 25 mL MeCN for 2 min, wash twice with 20 mL portions of MeCN, filter (cotton plug), wash filter with 30 mL n-hexane saturated with MeCN, add 30 g anhydrous sodium sulfate to the filtrate, let stand at room temperature for 30 min, filter (cotton plug), add 30 mL isopropanol to the filtrate. Evaporate the filtrate to dryness at 35°, reconstitute with 5 mL MeCN:MeOH 60:40, sonicate, add to the column, wash with 35 mL MeCN:MeOH 60:40, elute with 35 mL MeOH:water 75:25. Add 10 mL isopropanol to the eluate and evaporate it to dryness at 40°, reconstitute with 1 μg/mL chloramphenicol in MeCN:200 mM KH_2PO_4 15:85 containing 5 mM sodium 1-hexanesulfonate, filter (Gelman Ekikurodisk 13 CR), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 L-column ODS (Chemicals Inspection and Testing Institute, Tokyo)
Mobile phase: MeCN:10 mM pH 5.0 phosphate buffer 21:79
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 20.7
Internal standard: chloramphenicol (18.8)
Limit of detection: 3 ng/g

OTHER SUBSTANCES

Extracted: N^4-acetyl sulfaquinoxaline
Simultaneous: acetylsulfadiazine, acetylsulfadimethoxine, acetylsulfamethazine, acetylsulfamethoxazole, acetylsulfamonomethoxine, amprolium, diaveridine, ethopabate, furazolidone, nalidixic acid, nitrofurazone, ormetoprim, oxolinic acid, pyrimethamine, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, thiamphenicol, trimethoprim

KEY WORDS

chicken; muscle; liver; kidney; skin; plasma; SPE

REFERENCE

Takahashi,Y.; Sekiya,T.; Nishikawa,M.; Endoh,Y.S. Simultaneous high-performance liquid chromatographic determination of amprolium, ethopabate, sulfaquinoxaline, and N4-acetylsulfaquinoxaline in chicken tissues, *J.Liq.Chromatogr.*, **1994**, *17*, 4489–4512.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Add 300 μL acetone slowly while vortexing to 100 μL plasma, centrifuge at 1700 g for 4 min. Remove 100 μL of the supernatant and evaporate it to dryness under a stream of nitrogen at 65°, reconstitute the residue in 200 μL mobile phase, inject a 20-100 μL aliquot. Urine. 500 μL Urine + 500 μL 3 M pH 6 acetate buffer, mix, extract with 4 mL dichloromethane. Remove 0.5-1.5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 20-100 μL aliquot.

HPLC VARIABLES

Guard column: 40 × 3.2 30-40 μm Perisorb C18 (Merck)
Column: 250 × 4.6 10 μm Hibar II C18 (Merck)
Mobile phase: MeOH:200 mM pH 7 KH_2PO_4/Na_2HPO_4 35:65
Flow rate: 1.7
Injection volume: 20-100
Detector: UV 252 (plasma), UV 360 (urine)

CHROMATOGRAM

Retention time: 3.6
Limit of detection: 100 ng/mL (urine), 250 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites, N-acetylsulfaquinoxaline

KEY WORDS

rabbit; plasma; pharmacokinetics

REFERENCE

Eppel,J.G.; Thiessen,J.J. Liquid chromatographic analysis of sulfaquinoxaline and its application to pharmacokinetic studies in rabbits, *J.Pharm.Sci.*, **1984**, *73*, 1635–1638.

SAMPLE

Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 6.2
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

KEY WORDS
post-column reaction; meat; column-switching; dialysis

REFERENCE
Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 30:70
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 18.1
Limit of detection: 1.1 ppb
Limit of quantitation: 2.4 ppb

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadimethoxine, sulfamethazine

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing

100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 12.2
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole
Interfering: sulfadimethoxine (distinguish by MS)

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 13.09

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, , sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2

Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 18.05

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 56

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 65

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 16
Limit of detection: 0.01 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfadiazine, sulfamonomethoxine, sulfamethazine (sulfadimidine), sulfadimethoxine

KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue
Sample preparation: Cut tissue into small pieces and homogenize in blender. 20 g Homogenized tissue + 200 μL 10 μg/mL methyl p-aminobenzoate in water + 60 mL acetone:chloroform 50:50, shake vigorously on a mechanical shaker for 10 min, centrifuge at 3000 g for 10 min, filter (Whatman No. 41 paper) supernatant, repeat extraction. Combine the extracts, if the extract is not clear centrifuge at 3000 g for 10 min and discard the aqueous layer, evaporate to an oily residue at 45° under reduced pressure, add 5 mL MeCN to flask, let stand for 10 min, remove MeCN layer, add 5 mL hexane and 5 mL MeCN, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer, add 5 mL MeCN to the hexane layer, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer. If hexane layer is not clear centrifuge at 3000 g for 10 min and remove

the clear portion. Add 400 μL 15% trichloroacetic acid to the hexane layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Evaporate the MeCN layers, transfer the oily residue to a small flask with 3 mL hexane, add the aqueous trichloroacetic acid layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Discard the hexane layer, add 100 μL saturated aqueous sodium citrate solution to the aqueous layer, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP 18 (Brownlee)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 1% aqueous acetic acid. B was MeCN:water 80:20. A:B from 90:10 to 60:40 over 20 min, return to initial conditions over 5 min, re-equilibrate for 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 7 m × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 1 g p-dimethylaminobenzaldehyde in 30 mL MeCN, make up to 100 mL with 5% trichloroacetic acid in water.)

CHROMATOGRAM

Retention time: 26.0
Internal standard: methyl p-aminobenzoate (18.6)
Limit of detection: 30 ng/g

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfapyridine

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KEY WORDS

chicken; liver; pig; kidney; sheep; cow; post-column reaction

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REFERENCE

Bui,L.V. Liquid chromatographic determination of six sulfonamide residues in animal tissues using postcolumn derivatization, *J.AOAC Int.*, **1993**, *76*, 966–976.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The

effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 17
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; liver; kidney; SPE

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot. (Sodium acetate should be a highly pure grade.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 24
Limit of detection: 40 ng/g

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfamethazine

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL

water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

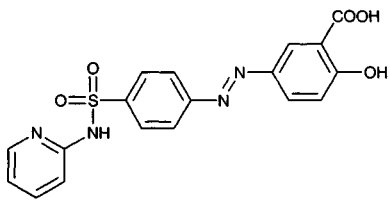
CHROMATOGRAM
Retention time: 24.5
Limit of quantitation: 5 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

Sulfasalazine



Molecular formula: $C_{18}H_{14}N_4O_5S$
Molecular weight: 398.40
CAS Registry No.: 599-79-1
Merck Index: 9112
Lednicer No.: 2 114

SAMPLE
Matrix: blood
Sample preparation: Mix 250 μL plasma with 500 μL 1 M HCl, extract with 4 mL ethyl acetate, evaporate to dryness under nitrogen at 40°, inject an aliquot.

HPLC VARIABLES
Column: 75 × 3.9 4 μm Nova-Pak LC-18
Mobile phase: MeOH:50 mM sodium acetate buffer 40:60
Flow rate: 0.5
Detector: UV 365

CHROMATOGRAM
Limit of quantitation: 100 ng/mL